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# The effect of some plant extracts on pathogenic fungi that cause human & animal infections

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Abstract:

Two types of plant extracts (Eucalyptus & Oleander)were evaluated for their antimicrobial activity against two food spoilage fungal organisms(Aspergillus flavus & Penecillum). The plant extracts were prepared by ethanolic extraction of their leaves & concentrated after that .Two concentrations of each extract were prepared (1,0.5)mg/ml& tested for their antifungal activity .

The results revealed that both extracts at the both concentrations inhibited the growth of (A. flavus & P.) significantly as compared with control .

For Eucalyptus extract ,the differences between (0.5& 1)mg/ml concentrations on both average diameter & inhibition zone was insignificant in their effect on( A. flavus & P.)but the differences were significant between only the tow concentrations & the control , For Oleander extract ,the differences between the two concentrations were insignificant on average diameter of both fungal organism ,as where on inhibition zone the differences were significant between the (1& 0.5)mg/ml concentrations ,the concentration 1mg/ml was more efficient than 0.5mg/ml concentration.

## Introduction

The infection with pathogenic fungi comprimize the high rate of humen diseases which include the cutaneous ,sub cutaneous & systemic diseases especially in the hot area which provide the suitable conditions that

enhanced the growing of such pathogenic fungi :as humidity ,high temperature & cheratinized material therefore the appearance of infectious fungi are widely disturbuted in our country (Abdelhamid *etal*,1999)

Once the food products contaminated with fungal microorganism existence of suitable temperature the fungus will use the food product as an energy source ,metabolizing it & excreting waste products such as toxins,(Jones,2000), may make the food product unpalatable & as aresult the food source is said to be spoiled at other times the waste products from fungal microorganism may cause food poisoning or food intoxications in the consumer (Alexopoulos & Mims,1979 ).

Both Aspergillus flavus & Penicillum are consider one of the most well known food poisoning fungal microorganism they grow perfectly on food product such as ( oranges, breads, rice & cereals other carbohydrated containing foods).which stored under bad condition in addition to that Aspergillus flavus release aflatoxins which causing hepatocarcinoma (WHO,1979),(Hadidane etal,1985).Because the plants containing a No. Of compounds made them of medical importance in treatment of series of diseases (Evans & Tayler,1992 ) . & that either by direct method by putting the intact plant part on the infected areas as using of myretus leaf in treatment of freckles the same thing with the using Ricinus communis in treatment of the dermatities .Drinking of apple tree powder effective in treatment of peptic ulcer (AL-Rawi ,1964).
Nerium Oleander this fast growing ever green shrub can reach up to feet tall it is tough ,versatile plant with showy summer time ,abundant ,beautiful flowers are produced in many colors & some varieties are delightfully fragrant ,is native to northern Africa ,the eastern Mediterranean basin & south east Asia ( Floridata,2004)
This species of plant is belong to Apocynaceae family it is consist of Neriodorein ,Neriline, Folinerin ,Rosaginine & Oleandrine(aglycoside) upon hydrolysis yields gitoxigenin, the uses of oleander is reported to be used in the treatment of heart diseases as a substitute for digitalis ,it is diuretic action is said to be very marked & not rarely it is acts as cathartic it is safer than digitalis in a theromatous diseases (Al-Rawi,1964&Goetz,1998).Eucalyptus is a tall ,ever green tree ,native to Australia & Tasmania successfully introduced world wide ( Bruneton,1995)&(Budavari,1996) the genus name Eucalyptus comes from the Greek word eucalyptos ,meaning "well –covered " (Grieve,1979) .Tough native to Australia, it is therapeutic uses have been introduced & integrated into traditional medicine, systems, including Chinese Indian Ayurvedic & Greco-European (Indian Pharmacopoeia,1996).Eucalyptus it is belong to Myrtaceae family & it is

of many classes they are E. coolabahs ,E. griffithii, E. incrassata , E. microtheca, E. rostrata &E. bicolor .its cultivated in any where & part of use is leaves & it is constituents are volatile oil ,containing cinol( Eucalyptol).Eucalyptol oil & eucalyptol produce a stimulating expectorant action when in contact with the membranes of the throat & the bronchial congestion .they are also mild anti inflammations of the mouth ,nose & throat(Al-Rawi,1964) .

The objective of present study was to evaluate the capability of some medicinal plants extracts to inhibit the growth of food spoilage fungal microorganism (Aspergillus flavus & Penicillium) .

## Materials & methods

**1-Collection & preparation of plants**

Plants samples leaves ( Eucalyptus bicolor & Nerium oleander) were collected from the garden of diyala medical college in October of 2005. after that drying was taken place on these leaves by oven at 60 C for 2 hrs (Toma,sada.1995) .Blending of these dried leaves was the next step & converted them into powder form finally these powders were stored in plastic containers till use .

**2- Preparation of alcoholic extract (70% ethanol conc.)**

the procedure for which included addition of leaves power (100 gm) in 500 ml of alcoholic solution ( conc. 70%) in a flask then this flask was shaking by shaker water bath at (40°C) for (18hrs) after that the solution was filtered by vacuum filtration & the resulted supernatant was concentrated by hot plate drying ,at( 60°C) till the concentrated solution became syrup or thick consistency & the final were in glass containers at ( 40°C) till use(Bruneton,1995).

**3- Experimental micro organisms**: Aspergillus flavus& Penicillium were used in this study to show the antifungal activity of plant extracts. They were isolated & diagnosed in the microbiology lab. Of medical college of diyala.

**4- Experimental plant species : Eucalyptus bicolor & Nerium oleander**(Al-Rawi,1964)(Grieve,1979)(Inchem,2005**).**

**5- Cultural medium**: Potato dextrose agar(PDA) ,sabouraud dextrose agar & semi solid PDA for culturing fungi. all these media were used according to the special directions of each one & they were sterilized by autoclave at 121°C for 15 min.

**6-Determination of antimicrobial activity** :

The chosen fungal organisms were prepared to the test ,The ethanolic extract of both ( Eucalyptus & oleander ) were mixed with (PDA) at final concentration of (1 &0.5)mg/ml then cultured with the Asprgillus flavus & Pencillum (two duplicate for each concentration ) and finally incubated at 25°C for 7 days . The diameter of growth (width & long) measured from the 3rd day of incubation to the 7th day . After that the inhibition zone for each culture plate were taken by the following equation .

$$\text{Inhibition zone} = \frac{\text{Diameter of control - Diameter of sample}}{\text{Diameter of sample}}$$

The data were analyzed statically (L.S.D)test at 0.05 level was used to compare between the means (Steel& Torry,1960).

## RESULTS &DISCUSSION:

Data revealed that both Eucalyptus & Oleander extracts have antifungal activity against Aspergillus flavus & Penicillium they inhibit their growth as compared with the control .As shown in table (1) the average diameter means of A. flavus & P. were (3.5- 3.925)cm respectively at concentration (0.5 mg /ml)of Eucalyptus extract whereas at concentration (1mg /ml) the means were ( 2.675, 2.975) cm respectively as compared with the control which has average diameter for A. flavus ( 7.75cm) & for P. ( 7.25cm) .

In addition to that Eucalyptus extract also has an obvious inhibition zone effect on A.flavus & P. growth demonstrated in table (1) .The inhibition zone means of Eucalyptus at concentration 0.5 mg/ml on A.flavus & P. were (1.33, 0.81) cm respectively while at (1mg /ml) were (1.85 , 1.43)cm respectively. These data indicate that as concentration of Eucalyptus extract increased the inhibition of growth will increased too. However its effect on inhibition zone at (o.5& 1) mg/ml is not significant but between the two concentrations & the control was significant according to the statical analysis.

These results agreed with Trivied & Hotchandani, 2004, who found that oil of Eucalyptus has the anti bacterial activity against gram positive as well as gram negative bacteria resistant to commonly used anti microbial agents since Eucalyptus ( 1,8-cineole) is the active ingredient of the Eucalyptus oil responsible for it is various pharmacological action(Indian pharmacopoeia,1996).

Table (2) demonstrate the effect of Oleander ethanolic extractant on the diameter means of A. flavus & P. ,at concentration (0.5 mg /ml) that (3.925 ,3.55)cm respectively where as at concentration (1mg/ml) were (2.975, 2,35)cm respectively too. Since the concentration (1mg/ml)was more effective than concentration (0.5mg/ml) ,but this effect was not significant. However these two concentrations was effective as compared with control.

In addition to the average diameter table 2 shown the effect of Oleander extract on the inhibition zone of A. flavus & P. growth , there is also an obvious & significant differences between the concentrations (1&0.5 )mg /ml .At concentration (0.5mg/ml)the inhibition zone means A. flavus & P. were (0.95, 2.04)cm respectively where as at (1 mg/ml) were ( 1.795 , 2.035)cm respectively.

From the above results it was obvious ,that Oleander extract has clear antifungal activity at both concentrations although the concentration (1 mg/ml) were more effective than (0.5 mg/ml).This activity may refer to the presence of glycosidic compound which is an active constituents (Gestetner *etal*,1970) &( Evans, 1992).

(Inchem ,2005) pointed out that Neriodorin ,a cardiac poison small doses in snake bites & powerful venomous bites,externally used in hamorroids cancers.

Table (3,4) Demonstrate the inhibition effect obviously on the growth and the average diameter of A. flavus & P. as compared with control group of both fungi.The same results thing with the Oleander extract which also inhibit the growth of both fungi for all period of growth as shown in table (4).These tables also revealed that between $5^{th}$ & $7^{th}$ days the average diameter of growth is nearly the same .(i.e.)the inhibitory effect act or it is effect appear from the beginning time of growth.

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Table (1)

Antifungal activity of Eucalyptus ethanolic extract on A.flavus &Penicillium

| | Concentration | Average diameter/ cm | means | Inhibition zone/cm | means |
|---|---|---|---|---|---|
| **Aspergillus flavus** | Control | 7.5 | 7.63 | | |
| | | 7.75 | | | |
| | 1mg/ml | 2.6 | 2.675 | 1.93 | 1.85 |
| | | 2.75 | | 1.77 | |
| | 0.5mg/ml | 3 | 3.5 | 1.54 | 1.23 |
| | | 4 | | 0.91 | |

| | | | | | |
|---|---|---|---|---|---|
| **L.S.D.** | | 1.35 | | 0.84 | |
| **Penicillium** | Control | 6.9 | 7.1 | | |
| | | 7.3 | | | |
| | 1mg/ml | 2.6 | 2.975 | 1.73 | 1.425 |
| | | 3.35 | | 1.12 | |
| | 0.5mg/ml | 4.1 | 3.925 | 0.73 | 0.81 |
| | | 3.75 | | 0.89 | |
| L.S.D. | | 1.19 | | 0.82 | |

Table (2)

Antifungal activity of Oleander ethanolic extract on A.flavus &Penicillium

| | Concentration | Average diameter/ cm | means | Inhibition zone/ cm | means |
|---|---|---|---|---|---|
| **Aspergillus flavus** | Control | 7.5 | 7.63 | | |
| | | 7.75 | | | |
| | 1mg/ml | 2.6 | 2.975 | 2.32 | 1.795 |
| | | 3.35 | | 1.27 | |
| | 0.5mg/ml | 4.1 | 3.925 | 0.86 | 0.95 |
| | | 3.75 | | 1.04 | |
| **L.S.D.** | | 1.48 | | 1.40 | |
| **Penicillium** | Control | 6.9 | 7.1 | | |
| | | 7.3 | | | |
| | 1mg/ml | 2.5 | 2.35 | 1.84 | 2.035 |
| | | 2.2 | | 2.23 | |
| | 0.5mg/ml | 3.4 | 3.55 | 1.09 | 2.01 |
| | | 3.7 | | 0.92 | |
| **L.S.D.** | | 0.81 | | 0.55 | |

Table (3)The effect of Eucalyptus extracts on the average diameter of A. flavus & Penicillium.

| days. / Fungal sp. | 3rd | 4th | 5th | 6th | 7th |
|---|---|---|---|---|---|
| A. flavus Control | 3.5- 4 | 4-5 | 5.5-6 | 8-7 | 8-7.5 |
| A.flavus Diameters/(cm) 1mg/ml | 1-0.7 | 1.3-1 | 2-1.5 | 2.5-2 | 2.7-2.5 |
| | 1-1 | 1.5-1.2 | 2-1.5 | 2.2-2.7 | 2.5-3 |
| A. flavus Diameters/(cm) 0.5mg/ml | 1.5-2 | 1.7-2.2 | 2.5-3 | 3.7-3 | 3-3 |
| | 2-2.5 | 2-2.8 | 2.5-3 | 3.5-4 | 4-4.2 |
| Penicillium | 2.5-2.3 | 4-3.8 | 4.8-4.5 | 6.4-6 | 7-6.8 |

| | | | | | |
|---|---|---|---|---|---|
| Diameters/(cm) Control | | | | | |
| Penicillium | 1-0.8 | 2-2 | 2-2 | 2-2.5 | 2.5-2.7 |
| Diameters/(cm) 1mg/ml | 1-1 | 1.2-1 | 1.3-1 | 1.3-1 | 1.4-1 |
| Penicillium | 1-1.3 | 1-1.5 | 2-1.5 | 2-1.5 | 2.2-2 |
| Diameters/(cm) 0.5 mg/ml | 1.5-2 | 2-1.5 | 2.7-2.2 | 2.8-2.5 | 3-2.5 |

Table (4) the effect of Oleander extracts on the average diameter of A . flavus & Penicillium.

| days. / Fungal sp. | 3rd | 4th | 5th | 6th | 7th |
|---|---|---|---|---|---|
| A. flavus Control | 3.5- 4* | 4-5 | 5.5-6 | 8-7 | 8-7.5 |
| A. flavus | 1- 0.8 | 2-2 | 2-2 | 2-2.5 | 2.5-2.7 |
| Diameters/(cm) 1mg/ml | 1-1 | 1.2-1 | 1.3-1 | 1.3-1 | 1.4-1 |
| A. flavus | 1-1.3 | 1-1.5 | 2-1.5 | 2-1.5 | 2.2-2 |
| Diameters/(cm) 0.5mg/ml | 1.5-2 | 2-1.5 | 2.7-2.2 | 2.8-2.5 | 3-2.5 |
| Penicillium Diameters/(cm) control | 2.5-2.3 | 4-3.8 | 4.8-4.5 | 6.4-6 | 7-6.8 |
| Penicillium | 1.3-1 | 2-1.5 | 2-1.5 | 2.5-2 | 2.8-2.2 |
| Diameters/(cm) 1mg/ml | 1-1 | 1.3-2 | 1.5-1.2 | 1.3-1.3 | 1.3-1.3 |
| Penicillium | 2-1.5 | 2.7-3 | 4-4.5 | 4.5-4.8 | 4.7-4.9 |
| Diameters/(cm) 0.5 mg/ml | 1.5-2 | 1.7-2.5 | 2.3-3 | 3-3.2 | 3.3-3.5 |

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# دراسة تأثير بعض مستخلصات النباتات على الفطريات التي قد تسبب أمراضا للإنسان والحيوان

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الملخص:

جربت الفعالية البيولوجية لنوعين من المستخلصات النباتية (الدفلة والكالبتوس) ضد نوعين من الفطريات المسببة لتلف الأغذية ( Aspergillus flavus ,Penicillium) 0 حضر المستخلصان النباتيان بطريقة الاستخلاص الكحولي لاوراقهما وتم تركيز هما للحصول على تركيزين (1و0,5 ) ملغم/مل .اظهرت النتائج ان كلا المستخلصات بكلا تركيز هما ثبطا نمو الفطرين (Aspergillus flavus,Penicillium) بصورة معنوية مقارنة بمجموعة السيطرة 0

فبالنسبة لتأثير مستخلص الكالبتوس الفرق بين التركيزين (1و0,5) ملغم/مل على كل من معدلات أقطار ودرجة التثبيط كان غير معنوي في تأثيره على كل من ( Aspergillus flavus, Penicillium ) لكن الفرق كان معنويا فقط بين كلا التركيزين أعلاه ومجموعة السيطرة 0 أما مستخلص الدفلة فان الفرق في التأثير بين التركيزين على معدلات أقطار نمو الفطرين(Aspergillus flavus, Penicillium ) كان بصورة غير معنوية بينما ظهر الفرق بين التركيزين في تاثيرهما على تثبيط نمو الفطرين أعلاه بصورة معنوية , حيث كان التركيز ( 1 ملغم /مل ) أكفا في التثبيط من التركيز (0,5ملغم/مل)0